

Q1
CONT.
for a time period sufficient to produce a culture having a compact multilayer
like appearance whereby said culturing is performed in the absence of a feeder
layer; and

(iii) identifying EG cells contained therein.

Sub 14. (Twice Amended) An improved method of producing chimeric avians which
comprises:

- Q2
(i) isolating primordial germ cells (PGCs) from an avian;
(ii) maintaining such PGCs in a tissue culture medium containing at least the
following growth factors;

- (1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and
(4) insulin-like growth factor (IGF)

for a sufficient time to produce embryonic germ (EG) cells whereby said
culture is maintained in the absence of a feeder layer;

- (iii) transferring said EG cells into a recipient avian embryo; and
(iv) selecting for chimeric avians which have the desired EG phenotype.

See the attached Appendix for the changes made to effect the above claim(s).

Please add the following new Claims 25-30:

25. An improved method of producing germline chimeric avians which comprises:

(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors;

(1) leukemia inhibitory factor (LIF),

(2) basic fibroblast growth factor (bFGF),

(3) stem cell factor (SCF) and

(4) insulin-like growth factor (IGF);

(iii) transferring said PGCs into a Stage XII-XIV recipient avian embryo; and

(iv) selecting for germline chimeric avians which have the desired PGC phenotype.

26. An improved method of producing germline or somatic cell chimeric avians

which comprises:

(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors;

(1) leukemia inhibitory factor (LIF),

(2) basic fibroblast growth factor (bFGF),

(3) stem cell factor (SCF) and

(4) insulin-like growth factor (IGF),

for a sufficient time to produce embryonic germ (EG) cells;

(iii) identifying and isolating embryonic germ (EG) cells from said cultured population of primordial germ cells;

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- (iv) transferring said isolated EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
 - (v) allowing said recipient avian to develop into a bird; and
 - (vi) selecting for germline or somatic cell chimeric avians that express the PGC phenotype.

27. A method for producing avian embryonic germ (EG) cells comprising the following steps:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer sufficient to produce a culture having a compact multilayer like appearance; and
- (iii) identifying the EG cells contained therein.

28. A method for producing chimeric avians comprising:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
- (iii) transferring said purified PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (iv) allowing said recipient avian to develop into a bird; and
- (v) selecting for chimeric avians that express the PGC phenotype.

29. A method for producing germline chimeric avians comprising:
- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
 - (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
 - (iii) transferring said purified PGCs into a recipient Stage XII-XIV avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
 - (iv) allowing said recipient avian to develop into a bird; and
 - (v) selecting for germline chimeric avians that express the PGC phenotype.

30. (New) A method for producing germline or somatic cell chimeric avians comprising:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
- (iii) identifying and isolating embryonic germ (EG) cells from said cultured population of primordial germ cells;
- (iv) transferring said isolated EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (iv) allowing said recipient avian to develop into a bird; and
- (v) selecting for germline or somatic cell chimeric avians that express the PGC phenotype.